

# Osteoarthritis and Cartilage



## Regional variation in T1ρ and T2 times in osteoarthritic human menisci: correlation with mechanical properties and matrix composition

M. Son †, S.B. Goodman †‡, W. Chen §, B.A. Hargreaves ||, G.E. Gold †‡||, M.E. Levenston †¶\*

† Department of Bioengineering, Stanford University, Stanford, CA, USA

‡ Department of Orthopaedic Surgery, Stanford University, Stanford, CA, USA

§ GE Healthcare, Menlo Park, CA, USA

|| Department of Radiology, Stanford University, Stanford, CA, USA

¶ Department of Mechanical Engineering, Stanford University, Stanford, CA, USA

### ARTICLE INFO

#### Article history:

Received 30 October 2012

Accepted 1 March 2013

#### Keywords:

Meniscus

T1ρ

T2

Biomechanics

Osteoarthritis

Degeneration

### SUMMARY

**Objective:** Changes in T1ρ and T2 magnetic resonance relaxation times have been associated with articular cartilage degeneration, but similar relationships for meniscal tissue have not been extensively investigated. This work examined relationships between T1ρ and T2 measurements and biochemical and mechanical properties across regions of degenerate human menisci.

**Design:** Average T1ρ and T2 relaxation times were determined for nine regions each of seven medial and 13 lateral menisci from 14 total knee replacement patients. Sulfated glycosaminoglycan (sGAG), collagen and water contents were measured for each region. Biomechanical measurements of equilibrium compressive, dynamic compressive and dynamic shear moduli were made for anterior, central and posterior regions.

**Results:** T1ρ and T2 times showed similar regional patterns, with longer relaxation times in the (radially) middle region compared to the inner and outer regions. Pooled over all regions, T1ρ and T2 times showed strong correlations both with one another and with water content. Correlations with biochemical content varied depending on normalization to wet or dry mass, and both imaging parameters showed stronger correlations with collagen compared to sGAG content. Mechanical properties displayed moderate inverse correlations with increasing T1ρ and T2 times and water content.

**Conclusion:** Both T1ρ and T2 relaxation times correlated strongly with water content and moderately with mechanical properties in osteoarthritic menisci, but not as strongly with sGAG or collagen contents alone. While the ability of magnetic resonance imaging (MRI) to detect early osteoarthritic changes remains the subject of investigation, these results suggest that T1ρ and T2 relaxation times have limited ability to detect compositional variations in degenerate menisci.

© 2013 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

### Introduction

The knee menisci distribute and transmit load through the joint by increasing the contact area of the tibiofemoral articulation, performing essential biomechanical functions<sup>1,2</sup>. The semi-lunar, fibrocartilaginous meniscus is mainly composed of water, multiple collagen types and proteoglycans (PGs)<sup>1,3</sup>. As in articular cartilage, negatively charged sulfated glycosaminoglycan (sGAG) side

chains attached to the PG core proteins osmotically attract water, contributing to the tissue's resistance to compression<sup>3</sup>. In early-stage osteoarthritis (OA), the meniscal extracellular matrix (ECM) exhibits increased sGAG and collagen contents, accompanied by increased swelling<sup>4</sup>. However, with further degeneration, the water content increases while PG and collagen contents decrease, resulting in inferior mechanical properties<sup>5,6</sup>. Such degenerative changes predispose the meniscus to injury and often precede cartilage degeneration during OA progression<sup>7,8</sup>. Thus, methods to detect changes that occur within the meniscus in a quantitative manner could provide powerful diagnostic and research measurements for early-stage OA. Recently, quantitative magnetic resonance imaging (MRI) parameters such as T1ρ and T2 relaxation times have been proposed to perform such functions and have been used to study compositional and structural changes in soft tissues.

\* Address correspondence and reprint requests to: M.E. Levenston, Department of Mechanical Engineering, Stanford University, 233 Durand Building, Stanford, CA 94305-4038, USA. Tel: 1-650-723-9464; Fax: 1-650-725-1587.

E-mail addresses: [minsunson@gmail.com](mailto:minsunson@gmail.com) (M. Son), [goodbone@stanford.edu](mailto:goodbone@stanford.edu) (S.B. Goodman), [weitian.chen@ge.com](mailto:weitian.chen@ge.com) (W. Chen), [bah@stanford.edu](mailto:bah@stanford.edu) (B.A. Hargreaves), [gold@stanford.edu](mailto:gold@stanford.edu) (G.E. Gold), [levenston@stanford.edu](mailto:levenston@stanford.edu) (M.E. Levenston).

T1 $\rho$  describes the relaxation time due to spin–lattice interactions in the rotating frame and has been shown to be sensitive to hydrogen exchange between slow macromolecules and bulk water<sup>9,10</sup>. Many have proposed that T1 $\rho$  can detect PG loss in cartilage by probing the interactions between the amine and hydroxyl groups on the GAG chains and water molecules<sup>10–12</sup>. Less energy exchange occurring between these macromolecules and water will result in longer T1 $\rho$  relaxation times. T2, on the other hand, measures the decay caused by spin–spin interactions and has been associated with hydration and collagen content and organization<sup>13–15</sup>. Collagen matrix degradation increases water mobility, decreasing the spin–spin interactions and increasing the T2 relaxation time. Mechanical properties are also affected by compositional and structural changes in the ECM, and in cartilage, T1 $\rho$  and T2 have shown negative correlations with various mechanical measures<sup>16,17</sup>.

As a result of their association with ECM changes, T1 $\rho$  and T2 changes in cartilage and meniscus have been associated with OA. In cartilage, both imaging parameters were found to increase with progressive stages of OA<sup>18,19</sup>, and significant correlations have been found between T2 and clinical symptoms<sup>20</sup>. In the meniscus, average T1 $\rho$  and T2 values were significantly elevated in mild and severe OA groups relative to healthy subjects<sup>21</sup>, T1 $\rho$  was observed to be greater in the lateral meniscus of anterior cruciate ligament (ACL)-injured patients compared to control patients<sup>22</sup> and T2 was found to correlate with Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores of OA severity<sup>23</sup>. T2\* imaging, which reflects relaxation due to magnetic field inhomogeneities in addition to the spin–spin interactions, has been explored as an approach to detect subclinical meniscal damage that manifests as changes in collagen structure<sup>24</sup>. While these quantitative imaging parameters have potential to improve clinical OA diagnosis, the sensitivity to regional variations remains unclear and the underlying mechanisms that cause changes in meniscal relaxation times are not completely understood.

Numerous studies have examined relationships between cartilage composition and T1 $\rho$  and T2 relaxation times, but similar in-depth studies on the meniscus have not yet been reported. Compared to cartilage, the meniscus is difficult to image due to its short T2 relaxation time, on the order of 8–18 ms<sup>23,25</sup>, which causes rapid transverse signal decay. The complex, heterogeneous regional patterns of tissue composition and mechanical function pose another challenge in the meniscus, requiring higher resolution to detect these differences. The radially inner, avascular region experiences high compressive stresses under functional loading and has a relatively high PG content<sup>26</sup>. The radially outer, vascularized region experiences high circumferential tension and has a relatively low PG content with collagen fiber bundles oriented primarily in the circumferential direction<sup>1</sup>. In addition, differences in the shape of the meniscus and position within the knee between medial and lateral menisci and anterior and posterior horns contribute to regional differences in loading<sup>27</sup>. The heterogeneous nature of the meniscus adds another layer of complexity in understanding OA progression, as the disease can affect one region differently than another.

In order to evaluate the potential of T1 $\rho$  and T2 measurements in the meniscus as potential diagnostic tools for OA, it is critical to understand what these relaxation times can detect about the degenerative state of the meniscus. The purpose of this study was to determine regional variations of T1 $\rho$  and T2 relaxation times in the radial and circumferential directions in OA human menisci, and to investigate their relations to biochemical contents (sGAG, collagen and water) and mechanical properties (equilibrium compressive, dynamic compressive and dynamic shear moduli). We hypothesized that regional variations in T1 $\rho$  and T2 times would reflect regional variations in tissue composition and

functional properties, with increased T1 $\rho$  and T2 in regions with greater water content and lower sGAG content, collagen content and mechanical properties.

## Methods

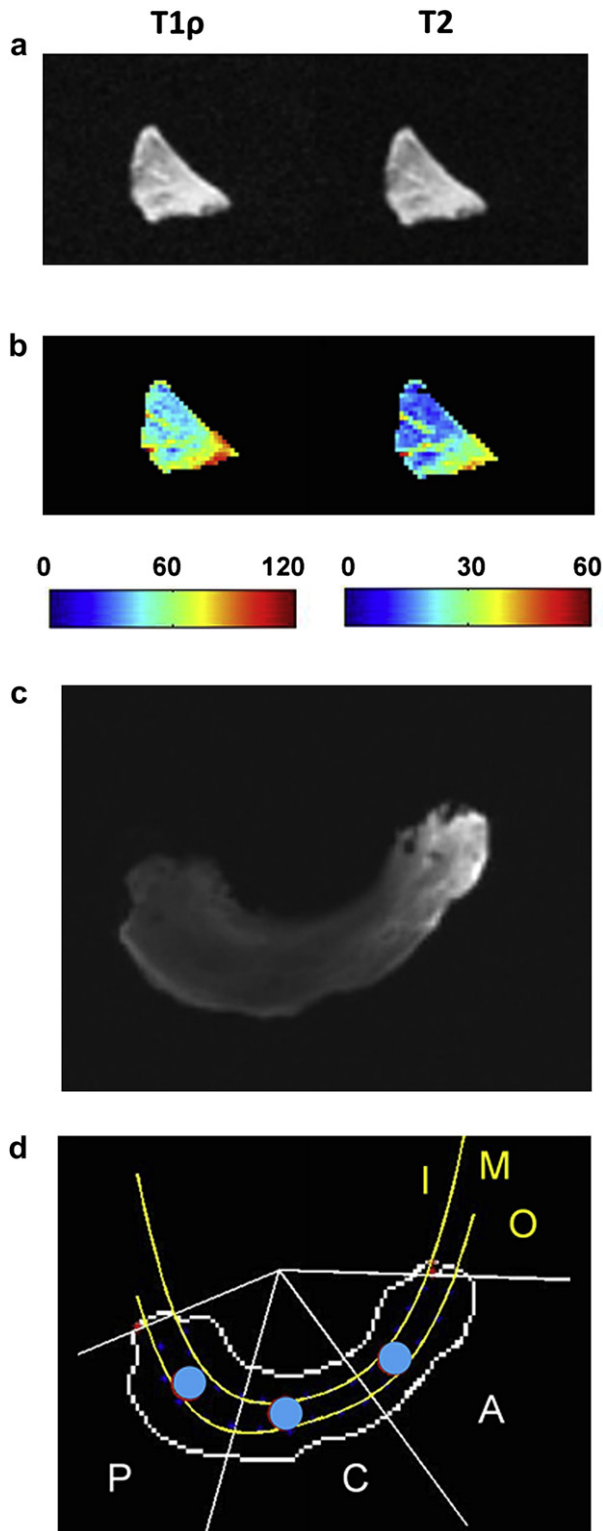
### Sample preparation

Twenty isolated menisci (13 lateral, seven medial) were obtained as incidental surgical waste from 14 patients (six males, eight females, age 64.6 (57.4, 71.7) years) undergoing total knee replacement (TKR) procedures at Stanford University Hospital. The specimens were stored until imaging at 4°C in 1X phosphate buffered saline (PBS, Mediatech, Manassas, VA) with protease inhibitors (PI, Protease Inhibitor Cocktail Set I; EMD Biosciences, San Diego, CA).

### Imaging

Within 24 h of surgery, each specimen was secured in a container filled with perfluorooctyl bromide (Exflur Research Corporation, Round Rock, TX) to minimize image artifacts from the air–tissue interface. T1 $\rho$ - and T2-weighted images were acquired using a Magnetization-Prepared Angle-Modulated Partitioned *k*-Space Spoiled Gradient Echo Snapshots (3D MAPSS) sequence that has a magnetization preparation followed by an immediate RF-spoiled gradient recalled echo (SPGR) acquisition during transient signal evolution<sup>28</sup>. To minimize magic angle effects, the specimens were oriented as if a patient were being imaged in a feet first, supine position, with the main circumferential collagen fibers oriented perpendicular to the main magnetic field. Coronal images were acquired with a 3 T GE scanner (MR750, GE Healthcare, Waukesha, WI) in an eight-channel wrist coil. Imaging parameters were repetition time (TR) = 7.5 ms, field of view (FOV) = 10 cm, matrix = 256 × 256, bandwidth (BW) = ±31.25 kHz, number of excitations (NEX) = 1, and 1 mm slice thickness. To tailor the sequence for the short T2 meniscus, the pulse sequence was designed to achieve echo time (TE) of 3.6 ms for SPGR readout. T1 $\rho$ -weighted images were collected at six spin-lock durations (TSL) (0, 4, 12, 20, 40, and 72 ms) with spin-lock frequency 500 Hz, and T2-weighted images were collected at six T2-preparation times (0, 6.4, 12.8, 19.3, 38.5, and 64.2 ms) [Fig. 1(a)]. Each scan took about 30 min.

The menisci were additionally scanned using a 3D gradient echo (GRE) sequence to obtain an image in the transverse plane that displayed the entire meniscus, showing its curvature [Fig. 1(c)]. The imaging parameters for this sequence were TR = 7.2 ms, TE = 3.4 ms, flip angle (FA) = 30°, FOV = 14 cm, matrix = 256 × 256, BW = ±31.25 kHz, NEX = 3 and 1.5 mm slice thickness. This image was used to define nine different regions in the meniscus [Fig. 1(d)]. First, the center of a circle was defined by the curvature of the meniscus using the Taubin circle fit method<sup>29</sup> (MATLAB v7.12, MathWorks, Natick, MA). The angle that encompassed the meniscus from this center was split into circumferential thirds, defining the boundaries of the anterior (A), central (C) and posterior (P) regions. To divide the meniscus in the radial direction, 10 equally spaced radial lines were drawn from the center of the Taubin circle to the outer edge of the meniscus. For each line, the intersection of the radial line with the inner and outer edges of the meniscus were identified, and the distance between these two points was divided into thirds marking the inner/middle and middle/outer boundaries. A polynomial fit was performed through corresponding inner and outer points to define continuous boundaries of the inner (I), middle (M) and outer (O) regions [Fig. 1(d)].



**Fig. 1.** Representative T1 $\rho$  and T2 (a) images at the first echo (TE = 0) and (b) maps of relaxation times from mono-exponential fits. 3D Fast GRE images (c) were used to divide the meniscus radially (d) into Inner (I), Middle (M) and Outer (O) thirds and circumferentially into Anterior (A), Central (C) and Posterior (P) thirds, resulting in nine separate regions for imaging and biochemical analysis. Circles indicate locations of mechanical testing samples.

From the 3D MAPSS sequence, each coronal slice was manually segmented and pixel intensities vs time for different TSLs and TEs were fit with mono-exponential decay equations (MATLAB) to obtain the T1 $\rho$  and T2 time constants for each pixel from each

region. T1 $\rho$  and T2 maps were generated for each slice [Fig. 1(b)], excluding boundary pixels to avoid partial volume effects. The regions identified in the 3D GRE images were mapped onto the cross-sectional T1 $\rho$ - and T2-weighted images and average relaxation times were calculated for each of these regions.

#### Biomechanical analysis

After imaging, the menisci were stored at  $-20^{\circ}\text{C}$  in PBS with proteinase inhibitors (EMD Millipore, Billerica, MA) and later thawed for biochemical and biomechanical analysis. 4 mm-diameter cores were taken from the radially middle portions of the anterior, central and posterior regions of each meniscus and were trimmed to 2 mm thickness, discarding the surface layers. Photographs of the meniscus were taken after the samples were cored in order to register the locations of the cores with the 3D GRE images. Briefly, the photograph and 3D GRE image were converted to binary images and the photograph image was iteratively transformed to minimize the error between the two images (MATLAB).

The samples were first tested at  $37^{\circ}\text{C}$  in oscillatory torsional shear using an AR-2000ex torsional rheometer (TA Instruments, New Castle, DE). Samples were compressed at 0.1%/s to 10%, allowed to stress relax for 20 min, and subjected to  $\pm 0.5\%$  nominal shear strain at 0.1 Hz to determine the dynamic shear modulus ( $G^*$ ). After unloading and recovery in PBS for 30 min, the samples were tested at room temperature in unconfined compression using an Instron 5848 MicroTester (Instron, Norwood, MA). Sequential compressive offsets of 5, 10, 15, and 20% were applied at 0.1%/s followed by stress relaxation for 20 min in order to obtain the equilibrium compressive modulus ( $E_{\text{eq}}$ ), which was determined via linear regression of the equilibrium stress vs applied strain as described previously<sup>30</sup>. At the 10% offset,  $\pm 1.5\%$  compressive strain was applied at 0.1 Hz to determine the dynamic compressive modulus ( $E^*$ ) based on Fast Fourier Transform analysis of the force data (MATLAB).

#### Biochemical analysis

Radial cross-sections with 1–2 mm thickness adjacent to the mechanical test samples were taken from the anterior, central, and posterior regions of the menisci and further divided into inner, middle and outer portions in roughly equal radial distances to obtain samples corresponding to all nine regions analyzed via MRI. Water contents were calculated using the wet and dry masses of the samples measured before and after lyophilization, respectively. The wet masses ranged from 10 to 50 mg, with a mean value of 32.5 mg (30.6, 34.4). The samples were then digested in 3 mg/ml Proteinase-K solution (Invitrogen, Carlsbad, CA) at  $60^{\circ}\text{C}$  overnight, with 1 mg of Proteinase-K for every 20 mg of tissue. sGAG contents were measured with the 1,9-dimethylmethylene blue (DMMB) spectrophotometric assay at 525 nm<sup>31</sup>, using chondroitin sulfate C (Sigma Aldrich, St. Louis, MO) standards. Collagen content was estimated from hydroxyproline content using the *p*-dimethylaminobenzaldehyde assay with trans-4-hydroxy-L-proline (Sigma Aldrich) standards<sup>32</sup> and an assumed collagen to hydroxyproline mass ratio of 8.

#### Histology

Additional intact radial cross-sections from the anterior, central and posterior regions were fixed in 10% neutral buffered formalin at room temperature for 12 h, dehydrated through graded alcohols, cleared with a xylene substitute (Histo-Clear, Electron Microscopy Sciences, Hatfield, PA), and embedded in paraffin. Sections were cut at 5  $\mu\text{m}$  using a sliding microtome and mounted on slides. The

slides were stained for sGAG with Safranin-O (Sigma Aldrich) and Fast Green counterstain (Electronic Microscopy Sciences, Hatfield, PA).

### Statistical analysis

Regional differences were evaluated using general linear models (GLMs) with  $P < 0.05$  and Tukey's test for pairwise comparisons, treating the donor as a random variable (Minitab v16, Minitab Inc., State College, PA). Data were checked for normality using an Anderson-Darling test. T1 $\rho$ , T2, sGAG content, and mechanical property data showed non-normal distributions and were log-transformed prior to GLM analysis. Imaging and biochemical data were analyzed with side (medial vs lateral meniscus), radial location (inner/middle/outer) and circumferential location (anterior/central/posterior) as factors, while mechanical properties were analyzed with side and circumferential location as factors. For each outcome, an initial multifactor model with all interactions was sequentially reduced via elimination of non-significant terms ( $P > 0.05$ ) while maintaining a hierarchical model.

Association across all pooled samples among imaging parameters, biochemical contents and mechanical properties were examined via Spearman's correlation (SPSS v19, IBM Corp, Armonk, NY). Correlation analyses of mechanical properties with imaging parameters used the average T1 $\rho$  and T2 relaxation times in the registered core regions from which the mechanical test samples were taken [Fig. 1(d)]. The mechanical properties were additionally

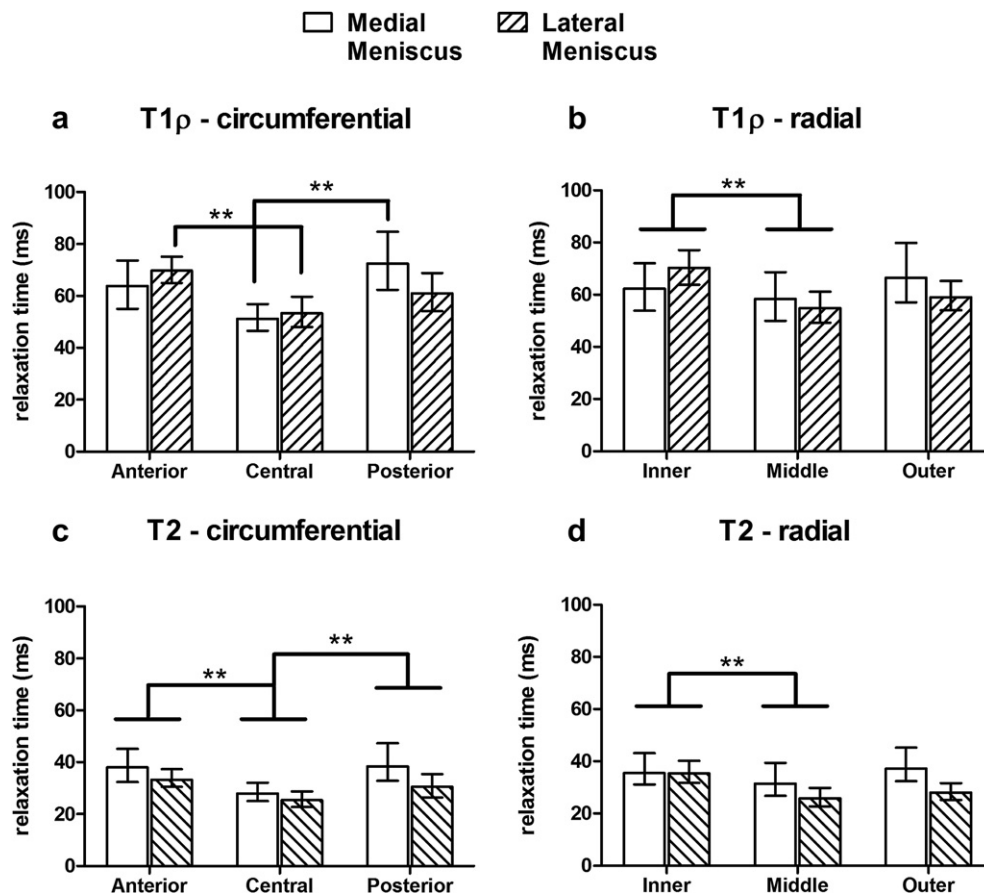
divided into upper and lower halves, and their correlations to T1 $\rho$  and T2 relaxation times were separately analyzed for the two subgroups.

### Results

#### Regional variation

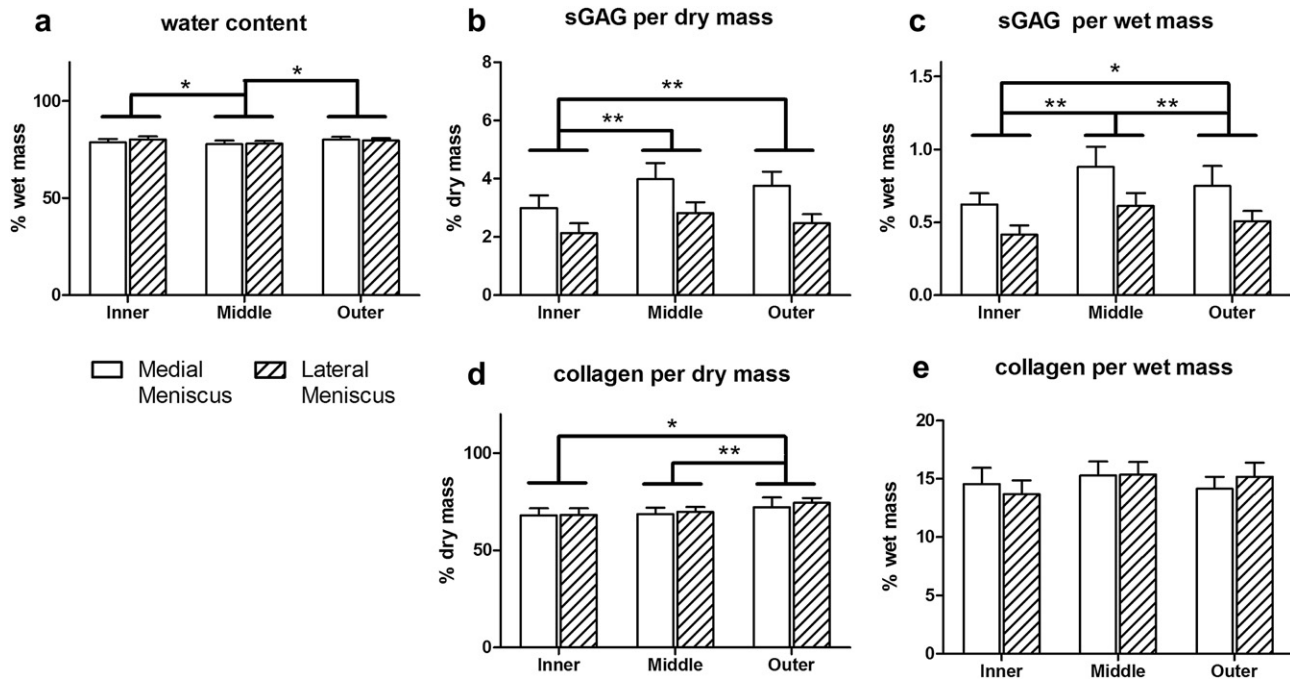
T1 $\rho$  and T2 relaxation times varied depending on region in both the radial and circumferential direction. The central region showed shorter T2 relaxation times than either the anterior ( $P < 0.0001$ ) or the posterior region ( $P = 0.0021$ ) [Fig. 2(c)]. T1 $\rho$  displayed similar patterns, with the lateral menisci showing lower relaxation times in its central vs anterior region ( $P = 0.0009$ ) and the medial menisci in its central vs posterior region ( $P = 0.0007$ ) [Fig. 2(a)]. In the radial direction, both relaxation times were lower in the middle region than in the inner region (T1 $\rho$ :  $P = 0.0043$ , T2:  $P = 0.0001$ ) [Fig. 2(b and d)]. Small mediolateral differences were observed in T2, which showed higher relaxation times in the medial meniscus ( $P = 0.0075$ ).

On the other hand, water content, sGAG per dry mass (dm) and collagen per dry mass did not show much variation among anterior, central, and posterior regions (sGAG:  $P = 0.52$ , collagen:  $P = 0.18$ , water:  $P = 0.56$ ) but varied in the radial direction. The outer and middle regions showed higher sGAG/dm than the inner region ( $O > I$ :  $P = 0.0056$ ,  $M > I$ :  $P < 0.0001$ ), and the outer region had higher collagen/dm than the other regions ( $I < O$ :  $P = 0.0017$ ,  $M < O$ :  $P = 0.0152$ ) [Fig. 3(b and d)]. Water content was lowest in the middle region ( $I > M$ :  $P = 0.031$ ,  $O > M$ :  $P = 0.013$ ) [Fig. 3(a)].



**Fig. 2.** Variation of (a and b) T1 $\rho$  and (c and d) T2 relaxation times with (a, c) circumferential and (b, d) radial location. Except for circumferential variation in the T1 $\rho$  time, the significance patterns were consistent for medial and lateral menisci. Both imaging parameters showed shorter relaxation times in the central region compared to the anterior and posterior regions, and in the middle region compared to the inner region. Data shown as mean  $\pm$  95% confidence interval ( $n = 19$ – $20$ ). \* $P < 0.05$ , \*\* $P < 0.005$ .

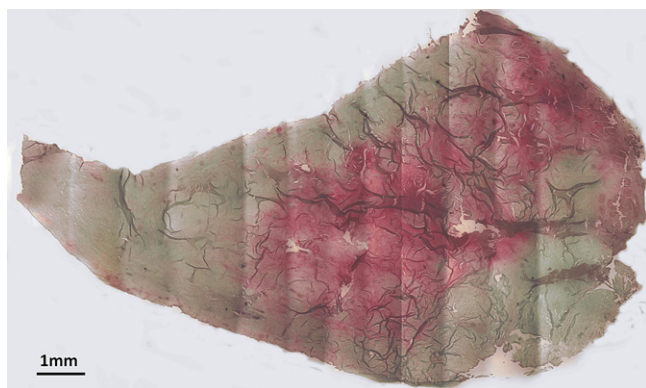




**Fig. 3.** Radial patterns of (a) water (b and c) sGAG and (d and e) collagen contents in OA human menisci. sGAG content showed the most radial variation with the inner region showing the lowest level of sGAG/dry mass (b) and sGAG/wm (c). Data shown as mean  $\pm$  95% confidence interval ( $n = 19$ – $20$ ). \* $P < 0.05$ , \*\* $P < 0.005$ .

Mediolateral differences in composition were also detected, with medial menisci showing higher sGAG/dm ( $P < 0.0001$ ) and lower collagen/dm ( $P = 0.0041$ ) and water content ( $P = 0.047$ ).

sGAG and collagen contents normalized by wet mass (wm) showed slightly different patterns. Collagen/wm showed little regional variation (medial vs lateral:  $P = 0.66$ , circumferential:  $P = 0.24$ , radial:  $P = 0.073$ ). sGAG/wm was higher in the medial meniscus than in the lateral meniscus for the anterior and central regions (A:  $P < 0.0001$ , C:  $P < 0.0001$ ) but not the posterior region ( $P = 0.11$ ). However, sGAG/wm did not exhibit significant circumferential variation in either medial ( $P \geq 0.12$ ) or lateral ( $P \geq 0.63$ ) menisci. sGAG/wm was highest in the radially middle region, followed by the outer and inner regions (M > O:  $P = 0.001$ , O > I:  $P = 0.023$ , M > I:  $P < 0.0001$ ) [Fig. 3(c)]. Safranin-O staining for sGAGs was consistent with the DMMB assay, with the greatest intensity in the middle region and the least in the inner regions (Fig. 4).

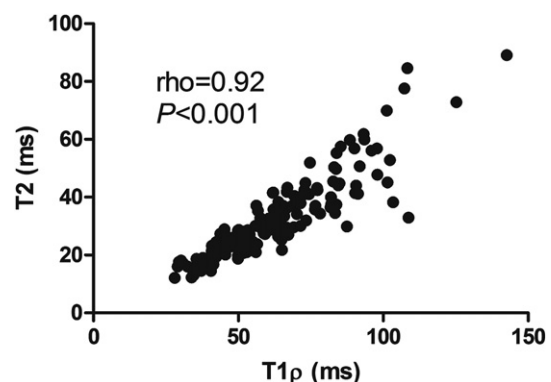


**Fig. 4.** Representative Safranin-O stained meniscal cross-section from the central region created by tiling 50 images. Red staining indicates regions of higher sGAG content. Consistent with quantitative measurements of sGAG content (Fig. 3), staining was most intense in the radially middle region and least intense in the radially inner region.

The average values for  $E_{eq}$ ,  $E^*$  and  $G^*$  were 52.2 (39.9, 72.6) kPa, 212.3 (157.8, 282.3) kPa, and 15.5 (12.0, 20.1) kPa, shown as mean (95% upper limit, 95% lower limit) respectively. Overall, mechanical properties exhibited little regional variation. No differences were detected for  $E_{eq}$  (medial vs lateral:  $P = 0.51$ , circumferential:  $P = 0.24$ ). The GLM indicated a significant interaction between side and circumferential location for  $E^*$  ( $P = 0.042$ ) and  $G^*$  ( $P = 0.031$ ), but none of the pairwise comparisons revealed differences ( $E^*$ :  $P \geq 0.25$ ,  $G^*$ :  $P \geq 0.20$ ).

#### Correlations

In addition to exhibiting similar regional patterns,  $T1\rho$  and  $T2$  relaxation times showed comparable correlations with biochemical contents and mechanical properties, and a high correlation was observed between the two imaging parameters ( $\rho = 0.92$ ,  $P < 0.001$ , Fig. 5). Both exhibited moderately strong correlations



**Fig. 5.**  $T2$  relaxation time plotted vs  $T1\rho$  relaxation time for OA human menisci, along with results of Spearman's correlation analyses. Pooled across all nine regions of all samples, a high positive correlation was found between  $T1\rho$  and  $T2$  relaxation times in OA human menisci.

with water content and with sGAG and collagen contents normalized to wet mass [Figs. 6 and 7]. However, collagen/dm showed a weak correlation with T2 relaxation time [Fig. 7(d)] and no correlation with T1 $\rho$  [Fig. 6(d)], and sGAG/dm did not correlate with either T1 $\rho$  or T2 time. As expected, water content showed strong inverse correlation with sGAG/wm and collagen/wm but did not correlate with sGAG or collagen normalized to dry mass (Table I). Overall, these results indicate that water content is a dominant determinant of T1 $\rho$  and T2 times in OA menisci.

All mechanical properties exhibited significant, negative correlations with both T1 $\rho$  and T2 relaxation times (Fig. 8). Interestingly, most of the variation in imaging parameters was seen among samples with lower moduli; when samples with low and high moduli were separately analyzed, correlation significance was either completely lost or substantially reduced. Similar to the imaging parameters, mechanical properties exhibited moderately strong correlations with water content, negative correlations with collagen/wm but none with collagen/dm (Table II). No correlations of mechanical properties with sGAG content were observed other than a moderately positive correlation between sGAG/wm and  $G^*$  (Table II). As with the imaging parameters, these results suggest that water content is a strong determinant of compressive and shear moduli in OA menisci.

## Discussion

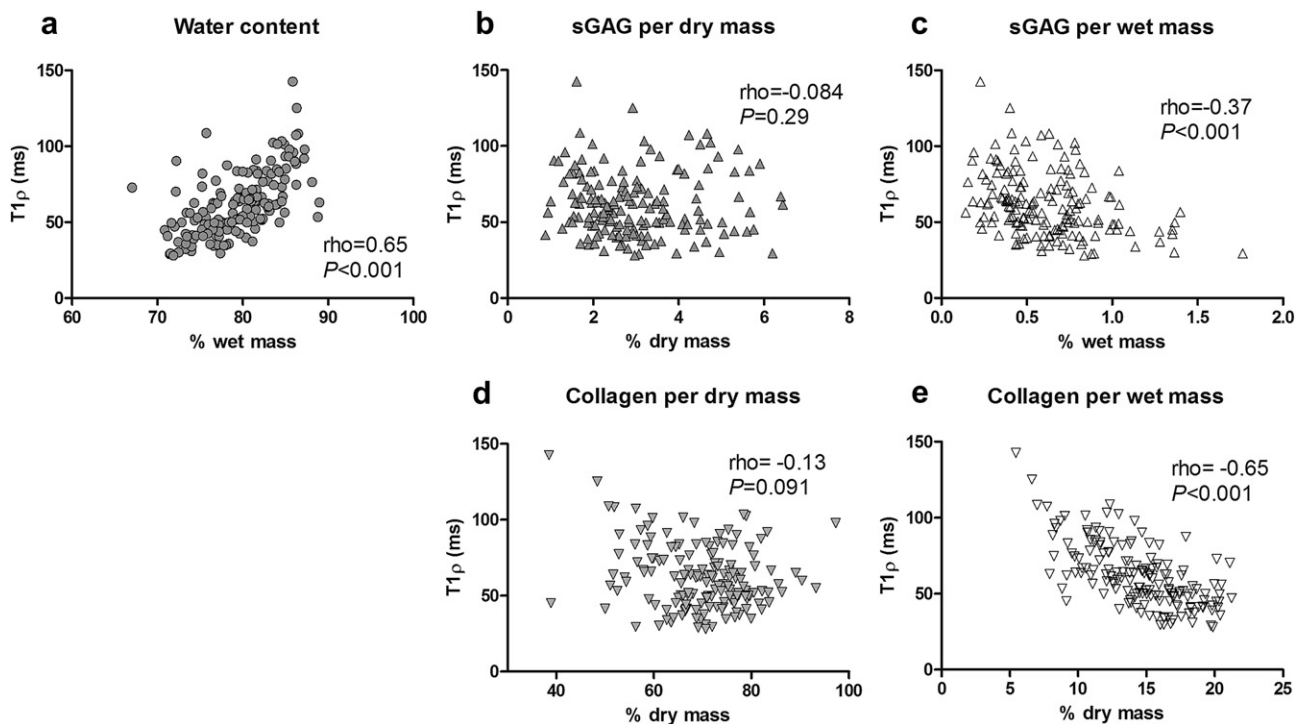
In this study, we explored regional variations of T1 $\rho$  and T2 relaxation times in degenerate human menisci and their relationships to biochemical contents and mechanical properties. We found that T1 $\rho$  and T2 times overall exhibited very similar regional patterns and were correlated with biochemical content and mechanical properties. However, the results suggested that variation in water content, rather than variations in sGAG or collagen contents,

determined much of the variation in T1 $\rho$  and T2 times in these samples.

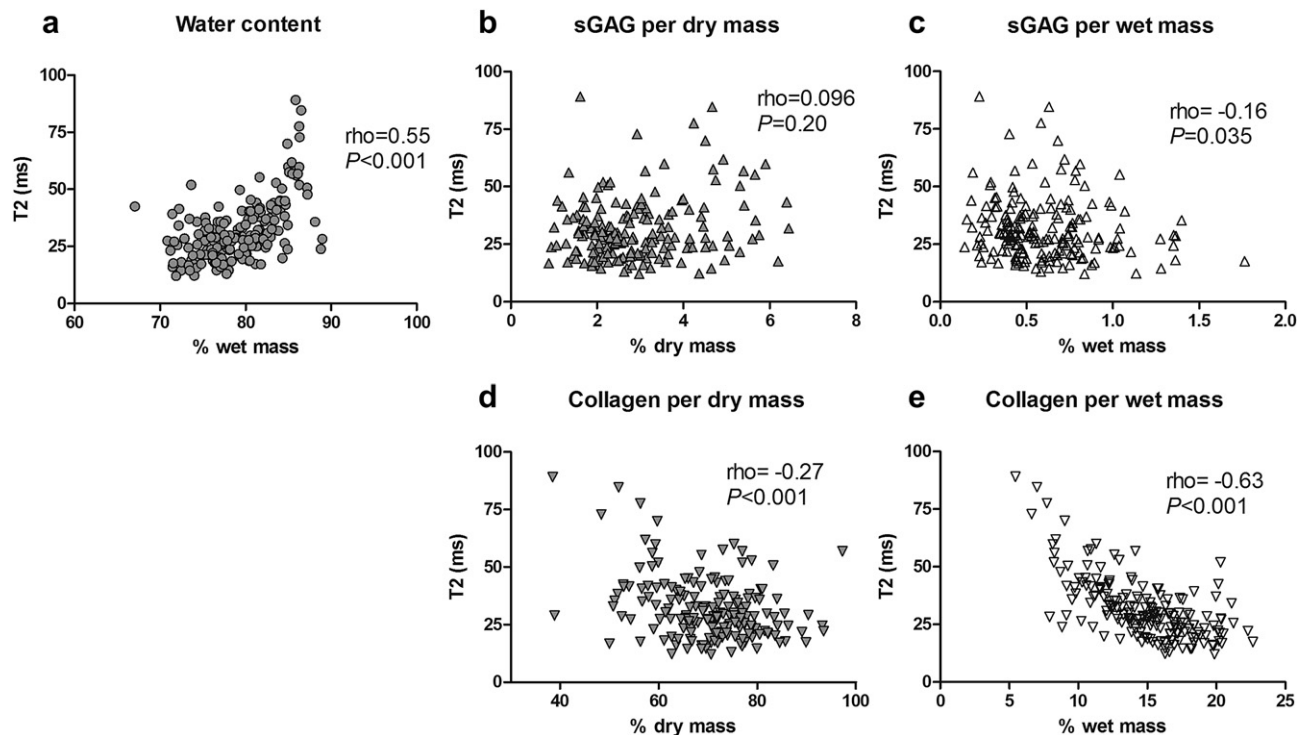
The T1 $\rho$  and T2 times observed in this study were consistently higher than previously reported *in vivo* values for the meniscus<sup>25,33</sup>. This difference could be due to the fact that we used severely degenerated menisci from TKR patients, as diseased tissue has been reported to have higher relaxation times<sup>21–23</sup>. Additionally, the measurements were taken *ex vivo*, necessitating storage in PBS prior to imaging, and changes in tissue hydration during this period may also contribute to the prolonged signal. However, as the purpose of this study was to examine the regional patterns within the meniscus and the relationships between the imaging and physical parameters, the absolute values of the time constants are not crucial in the interpretation of our results. All specimens were subject to a constant experimental procedure, and the relationships among the variables examined are expected to remain valid in this context.

Few studies have investigated regional variations of T1 $\rho$  or T2 relaxation times in the meniscus. Tsai *et al.* found that T2 relaxation times gradually increased from the inner to the outer regions in healthy human menisci<sup>25</sup>. We found an opposite pattern in OA menisci, with lower time constants in the middle region compared to the inner region. This altered pattern is consistent with the altered matrix composition in highly degenerated menisci. Although the inner region of the normal meniscus has the highest PG content<sup>26,34</sup>, the PG content of the middle region increases with age and the onset of OA<sup>35,36</sup>. We found low sGAG contents in the inner regions of our meniscal samples, suggesting that substantial PG degradation had occurred by late-stage OA. As shown by the DMMB assay and Safranin-O stained sections, PG content was highest in the middle region, which also showed the shortest T1 $\rho$  and T2 relaxation times.

In cartilage, many studies have reported specific associations of T1 $\rho$  with sGAG content and of T2 with collagen content and



**Fig. 6.** T1 $\rho$  relaxation time plotted vs composition of OA human menisci, along with results of Spearman's correlation analyses. Pooled across all regions of all samples, T1 $\rho$  relaxation time displayed a positive correlation with water content (a), no correlation with sGAG (b) or collagen (d) per dry mass, and negative correlations with sGAG (c) and collagen (e) per wet mass.



**Fig. 7.** T2 relaxation time plotted vs composition of OA human menisci, along with results of Spearman's correlation analyses. Pooled across all regions of all samples, T2 relaxation time displayed a positive correlation with water content (a), no correlation with sGAG per dry mass (b) and a negative correlation with collagen per dry mass (d), and negative correlations with sGAG (c) and collagen (e) per wet mass.

organization<sup>12,13</sup>. However, few of these studies separately reported the biochemical contents per wet and dry mass. In our study, we saw substantial differences between the two normalizations. sGAG/wm showed relatively stronger correlation with T1 $\rho$  than with T2, similar to the findings in cartilage that T1 $\rho$  was more sensitive to PG content than was T2. However, we found no correlations between the imaging parameters and sGAG/dm. Additionally, we found stronger correlations with collagen/wm compared to sGAG/wm for both T1 $\rho$  and T2 (Figs. 6 and 7). As with sGAG, correlations between imaging parameters and collagen content were weaker or absent when normalized to dry mass. Combined with the direct correlations of both imaging parameters with water content, these results strongly suggest that water content may be the dominant factor in determining T1 $\rho$  and T2 relaxation times in highly degenerate menisci.

While T1 $\rho$  and T2 describe different magnetic resonance relaxation mechanisms, they both probe for the motion of water molecules, which are greatly influenced by changes in sGAG and collagen contents. The similar regional patterns and high correlation between the two imaging parameters are congruent with previous reports in cartilage. Mlynarik *et al.* found almost identical

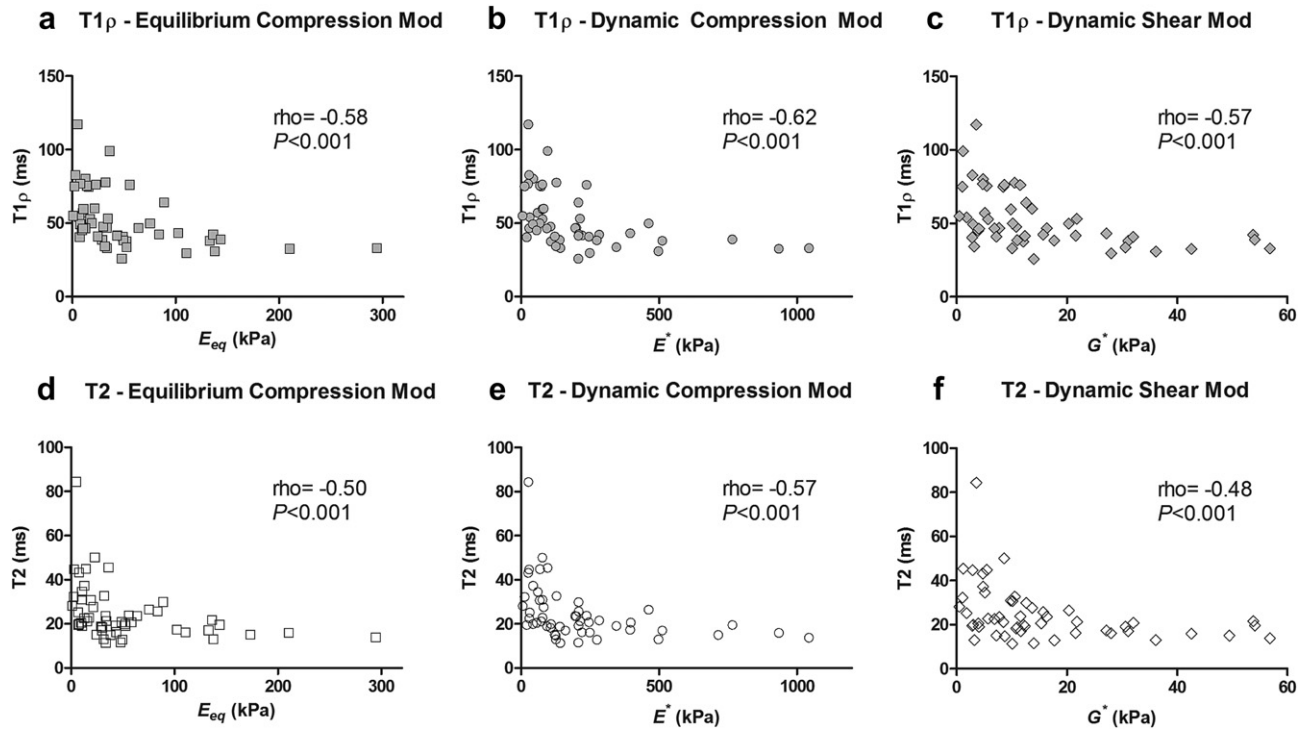
T1 $\rho$  and T2 map distributions in cartilage specimens obtained from total joint replacement surgeries<sup>37</sup>, and Majumdar *et al.* observed a significant, direct correlation between the two parameters *in vivo*<sup>38</sup>. These results indicate that, by this advanced stage of OA, any specific sensitivity T1 $\rho$  or T2 might have to a biochemical constituent in the meniscus is overwhelmed by their sensitivity to changes in water content. It should be noted that the meniscus has a much lower PG concentration than cartilage<sup>39</sup>, and the changes in PG content that may be possible to detect in cartilage by the T1 $\rho$  relaxation mechanism may not be detectable in the meniscus.

The influence of water on T1 $\rho$  and T2 relaxation times was also reflected in the imaging parameters' moderate correlations with  $E_{eq}$ ,  $E^*$  and  $G^*$ , which were in turn negatively correlated with water (Fig. 8, Table II). While the correlations between the imaging parameters and mechanical properties were moderately strong, this relationship did not hold in narrow ranges when samples with only high or low moduli were considered, indicating that T1 $\rho$  and T2 may not have high sensitivity to mechanical function. None of the measured mechanical properties showed much regional dependence, consistent with previous studies in older patients<sup>40</sup>. However, some other studies have shown regional variation in mechanical properties in relatively younger and healthier patients<sup>6,41</sup>. It will be interesting to investigate whether T1 $\rho$  and T2 reflect those regional patterns in that population, as it would indicate that these imaging parameters could differentiate between regions of weak and strong mechanical properties and perhaps detect changes in regional patterns earlier in OA development. It should be noted here that the measured mechanical properties may not be an accurate average of the entire region as the core sample preparation procedure itself disrupts the interaction between the collagen fibers, which contributes to the biomechanics of the tissue. While this is inevitable in mechanical testing of this nature, future studies may explore different methods such as indentation testing of an intact meniscus in order to avoid such limitations.

**Table I**

Spearman's correlations between measures of sGAG and collagen content and water content for OA human menisci, pooled across all regions of all samples. Neither sGAG nor collagen per dry mass were significantly correlated with water content, while (as expected) both sGAG and collagen per wet mass displayed significant, negative correlations with water content

	Water	
	Rho	P
sGAG per dry mass	-0.01	0.92
sGAG per wet mass	-0.43	<0.001
Collagen per dry mass	0.084	0.27
Collagen per wet mass	-0.81	<0.001



**Fig. 8.** T1 $\rho$  (a and c) and T2 (d and f) relaxation time plotted vs mechanical properties of OA human menisci, along with results of Spearman's correlation analyses. Pooled across all regions of all samples, both T1 $\rho$  (a and c) and T2 (d and f) relaxation time displayed similar, negative correlations with the equilibrium (a, d) and dynamic (b, e) unconstrained compression moduli and with the dynamic shear modulus (c, f). Note that samples with high moduli displayed minimal variation in either T1 $\rho$  or T2 relaxation times.

In this study, we found that the regional variation in T1 $\rho$  and T2 relaxation times reflect changes in biochemical composition, but are mostly associated with differences in water content. The association of imaging parameters with the composition and mechanical properties of the tissue indicate that T1 $\rho$  and T2 have the potential to be noninvasive markers of meniscal degeneration. However, we only examined menisci from late-stage OA patients in this study, and future work should include healthy, age-controlled samples in order to establish a baseline to properly interpret changes in T1 $\rho$  and T2 relaxation times. We need to investigate whether the correlations found in this study hold in earlier stages of OA, and whether these relationships are sensitive enough to detect subtle changes. Thus, understanding how the compositional and mechanical heterogeneity of the meniscus changes from a healthy, normal state to different stages of OA and investigating how these changes are reflected in the imaging parameters will be

essential in developing protocols for detecting early degenerative changes in the meniscus. This study is a starting point in evaluating T1 $\rho$  and T2 relaxation times as potential markers of meniscal degeneration and the first to investigate the relationship between these imaging parameters and the biochemical and mechanical properties of the meniscus.

#### Author contributions

Min-Sun Son contributed to the concept and design of the study, acquisition of data, analysis and interpretation of data and drafted the article. Dr. Chen provided the MAPSS sequence and technical support to run the study. Dr. Goodman provided the meniscal specimens used in the study and contributed to study design. Drs. Levenston, Hargreaves and Gold contributed to the concept and design of the study and analysis and interpretation of data. Drs. Levenston and Gold additionally provided funding for the study. All authors critically revised the article and approved the final version to be submitted. Min-Sun Son and Marc Levenston take responsibility for the integrity of this work as a whole and can be contacted at [minsunson@gmail.com](mailto:minsunson@gmail.com) and [levenston@stanford.edu](mailto:levenston@stanford.edu).

#### Competing interests

Weitian Chen is employed by GE Healthcare.

#### Role of the funding source

This research was supported by NIH R01AR052861, NIH R01EB002524, NIH K24AR062068, a Bio-X Graduate Fellowship, the Siebel Scholars Foundation, GE Healthcare, the Arthritis Foundation and SCBT-MR. None of the sponsors had any involvement in the design, execution or interpretation of the study or in the writing or submission of the manuscript.

**Table II**

Spearman's correlations between mechanical properties and biochemical composition of OA human menisci, pooled across all regions of all samples.  $E_{eq}$  is the equilibrium compression modulus,  $E^*$  is the dynamic compression modulus, and  $G^*$  is the dynamic shear modulus. All three mechanical properties showed strong, negative correlations with water content, while none of the properties were significantly correlated with either sGAG or collagen per dry mass. Only the shear modulus showed a significant, positive correlation with sGAG per wet mass, but all three properties showed significant, positive relationships with collagen per wet mass (likely reflecting the influence of water content)

	$E_{eq}$		$E^*$		$G^*$	
	Rho	P	Rho	P	Rho	P
Water	-0.59	<0.001	-0.60	<0.001	-0.61	<0.001
sGAG per dry mass	-0.053	0.71	-0.14	0.33	0.058	0.68
sGAG per wet mass	-0.20	0.15	0.14	0.33	0.34	0.014
Collagen per dry mass	-0.086	0.54	-0.048	0.73	-0.072	0.61
Collagen per wet mass	0.53	<0.001	0.54	<0.001	0.54	<0.001



## Acknowledgments

The authors thank Dr. Emily McWalter for her support in conducting experiments and interpreting data.

## References

- Messner K, Gao J. The menisci of the knee joint. Anatomical and functional characteristics, and a rationale for clinical treatment. *J Anat* 1998;193(Pt 2):161–78.
- Masouros SD, McDermott ID, Amis AA, Bull AM. Biomechanics of the meniscus-meniscal ligament construct of the knee. *Knee Surg Sports Traumatol Arthrosc* 2008;16:1121–32.
- McDevitt CA, Webber RJ. The ultrastructure and biochemistry of meniscal cartilage. *Clin Orthop Relat Res* 1990;252:8–18.
- Hellio Le Graverand MP, Vignon E, Otterness IG, Hart DA. Early changes in lapine menisci during osteoarthritis development: part I: cellular and matrix alterations. *Osteoarthritis Cartilage* 2001;9:56–64.
- Herwig J, Egner E, Buddecke E. Chemical changes of human knee joint menisci in various stages of degeneration. *Ann Rheum Dis* 1984;43:635–40.
- Bursac P, Arnoczky S, York A. Dynamic compressive behavior of human meniscus correlates with its extra-cellular matrix composition. *Biorheology* 2009;46:227–37.
- Lohmander LS, Englund PM, Dahl LL, Roos EM. The long-term consequence of anterior cruciate ligament and meniscus injuries: osteoarthritis. *Am J Sports Med* 2007;35:1756–69.
- Englund M, Lohmander LS. Risk factors for symptomatic knee osteoarthritis fifteen to twenty-two years after meniscectomy. *Arthritis Rheum* 2004;50:2811–9.
- Sepponen RE, Pohjonen JA, Sipponen JT, Tantt JI. A method for T1 rho imaging. *J Comput Assist Tomogr* 1985;9:1007–11.
- Duvvuri U, Goldberg AD, Kranz JK, Hoang L, Reddy R, Wehrli FW, et al. Water magnetic relaxation dispersion in biological systems: the contribution of proton exchange and implications for the noninvasive detection of cartilage degradation. *Proc Natl Acad Sci USA* 2001;98:12479–84.
- Wheaton AJ, Dodge GR, Borthakur A, Kneeland JB, Schumacher HR, Reddy R. Detection of changes in articular cartilage proteoglycan by T(1rho) magnetic resonance imaging. *J Orthop Res* 2005;23:102–8.
- Duvvuri U, Reddy R, Patel SD, Kaufman JH, Kneeland JB, Leigh JS. T1rho-relaxation in articular cartilage: effects of enzymatic degradation. *Magn Reson Med* 1997;38:863–7.
- Nieminen MT, Toyra J, Rieppo J, Hakumaki JM, Silvennoinen J, Helminen HJ, et al. Quantitative MR microscopy of enzymatically degraded articular cartilage. *Magn Reson Med* 2000;43:676–81.
- Nieminen MT, Rieppo J, Toyra J, Hakumaki JM, Silvennoinen J, Hyttinen MM, et al. T2 relaxation reveals spatial collagen architecture in articular cartilage: a comparative quantitative MRI and polarized light microscopic study. *Magn Reson Med* 2001;46:487–93.
- Lusse S, Claassen H, Gehrke T, Hassenpflug J, Schunke M, Heller M, et al. Evaluation of water content by spatially resolved transverse relaxation times of human articular cartilage. *Magn Reson Imaging* 2000;18:423–30.
- Wheaton AJ, Dodge GR, Elliott DM, Nicoll SB, Reddy R. Quantification of cartilage biomechanical and biochemical properties via T1rho magnetic resonance imaging. *Magn Reson Med* 2005;54:1087–93.
- Nissi MJ, Rieppo J, Toyra J, Laasanen MS, Kiviranta I, Nieminen MT, et al. Estimation of mechanical properties of articular cartilage with MRI – dGEMRIC, T2 and T1 imaging in different species with variable stages of maturation. *Osteoarthritis Cartilage* 2007;15:1141–8.
- Stahl R, Luke A, Li X, Carballido-Gamio J, Ma CB, Majumdar S, et al. T1rho, T2 and focal knee cartilage abnormalities in physically active and sedentary healthy subjects versus early OA patients – a 3.0-Tesla MRI study. *Eur Radiol* 2009;19:132–43.
- Stahl R, Blumenkrantz G, Carballido-Gamio J, Zhao S, Munoz T, Hellio Le Graverand-Gastineau MP, et al. MRI-derived T2 relaxation times and cartilage morphometry of the tibio-femoral joint in subjects with and without osteoarthritis during a 1-year follow-up. *Osteoarthritis Cartilage* 2007;15:1225–34.
- Dunn TC, Lu Y, Jin H, Ries MD, Majumdar S. T2 relaxation time of cartilage at MR imaging: Comparison with severity of knee osteoarthritis. *Radiology* 2004;232:592–8.
- Rauscher I, Stahl R, Cheng J, Li X, Huber MB, Luke A, et al. Meniscal measurements of T1rho and T2 at MR imaging in healthy subjects and patients with osteoarthritis. *Radiology* 2008;249:591–600.
- Bolbos RI, Link TM, Ma CB, Majumdar S, Li X. T1rho relaxation time of the meniscus and its relationship with T1rho of adjacent cartilage in knees with acute ACL injuries at 3 T. *Osteoarthritis Cartilage* 2009;17:12–8.
- Zarins ZA, Bolbos RI, Pialat JB, Link TM, Li X, Souza RB, et al. Cartilage and meniscus assessment using T1rho and T2 measurements in healthy subjects and patients with osteoarthritis. *Osteoarthritis Cartilage* 2010;18:1408–16.
- Williams A, Qian Y, Golla S, Chu CR. UTE-T2\* mapping detects sub-clinical meniscus injury after anterior cruciate ligament tear. *Osteoarthritis Cartilage* 2012;20:486–94.
- Tsai PH, Chou MC, Lee HS, Lee CH, Chung HW, Chang YC, et al. MR T2 values of the knee menisci in the healthy young population: zonal and sex differences. *Osteoarthritis Cartilage* 2009;17:988–94.
- Nakano T, Dodd CM, Scott PG. Glycosaminoglycans and proteoglycans from different zones of the porcine knee meniscus. *J Orthop Res* 1997;15:213–20.
- Jones RS, Keene GC, Learmonth DJ, Bickerstaff D, Nawana NS, Costi JJ, et al. Direct measurement of hoop strains in the intact and torn human medial meniscus. *Clin Biomech (Bristol, Avon)* 1996;11:295–300.
- Li X, Han ET, Busse RF, Majumdar S. In vivo T(1rho) mapping in cartilage using 3D magnetization-prepared angle-modulated partitioned k-space spoiled gradient echo snapshots (3D MAPSS). *Magn Reson Med* 2008;59:298–307.
- Taubin G. Estimation of planar curves, surface, and nonplanar space curves defined by implicit equations with applications to edge and range image segmentation. *IEEE Trans Patt Anal Mach Intell* 1991;13:1115–38.
- Palmer AW, Wilson CG, Baum EJ, Levenston ME. Composition-function relationships during IL-1-induced cartilage degradation and recovery. *Osteoarthritis Cartilage* 2009;17:1029–39.
- Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochim Biophys Acta* 1986;883:173–7.
- Woessner Jr JF. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch Biochem Biophys* 1961;93:440–7.
- Wang L, Chang G, Xu J, Vieira RL, Krasnokutsky S, Abramson S, et al. T1rho MRI of menisci and cartilage in patients with osteoarthritis at 3T. *Eur J Radiol* 2012;81:2329–36.
- Killian ML, Lepinski NM, Haut RC, Haut Donahue TL. Regional and zonal histo-morphological characteristics of the lapine menisci. *Anat Rec (Hoboken)* 2010;293:1991–2000.

35. Pauli C, Grogan SP, Patil S, Otsuki S, Hasegawa A, Koziol J, *et al.* Macroscopic and histopathologic analysis of human knee menisci in aging and osteoarthritis. *Osteoarthritis Cartilage* 2011;19:1132–41.
36. Chevrier A, Nelea M, Hurtig MB, Hoemann CD, Buschmann MD. Meniscus structure in human, sheep, and rabbit for animal models of meniscus repair. *J Orthop Res* 2009;27:1197–203.
37. Mlynarik V, Trattnig S, Huber M, Zembsch A, Imhof H. The role of relaxation times in monitoring proteoglycan depletion in articular cartilage. *J Magn Reson Imaging* 1999;10:497–502.
38. Majumdar S, Li X, Blumenkrantz G, Saldanha K, Ma CB, Kim H, *et al.* MR imaging and early cartilage degeneration and strategies for monitoring regeneration. *J Musculoskelet Neuronal Interact* 2006;6:382–4.
39. Adams ME, Muir H. The glycosaminoglycans of canine menisci. *Biochem J* 1981;197:385–9.
40. Leslie BW, Gardner DL, McGeough JA, Moran RS. Anisotropic response of the human knee joint meniscus to unconfined compression. *Proc Inst Mech Eng H* 2000;214:631–5.
41. Chia HN, Hull ML. Compressive moduli of the human medial meniscus in the axial and radial directions at equilibrium and at a physiological strain rate. *J Orthop Res* 2008;26:951–6.